



Valorization of amazonian fruit agro-industrial by-products for sustainable production of bacterial cellulose nanofibers for food bio-packaging

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Abstract

The expansion of agro-industry creates large volumes of carbon-rich organic waste that pose environmental challenges but also offer opportunities for valorization as sustainable feedstocks for biocomposite production. This study aimed to produce and characterize bacterial cellulose nanofibers (BCN) from agro-industrial wastes (AIW) from soursop (*Annona muricata* L.; BCN-S) and genipap (*Genipa americana* L.; BCN-G) collected in the Brazilian Amazon. Substrates (2.5% w/v) were heated to 70 °C for 15 min and inoculated with 10% (w/v) starter culture SCOBY (symbiotic culture of bacteria and yeasts). The substrates exhibited variations in the carbohydrate-to-cellulose conversion profile due to the selective activity of SCOBY. BCN-G showed a good yield (14.50 g/L), highest crystallinity index (CrI=75.2%), a calculated crystallite size (D_{av}) of 163.1 nm and the presence of type I cellulose, as well as traces of type II cellulose. However, the control BCN sample (sucrose) exhibited a higher enthalpy of fusion (280 J/g), suggesting greater crystalline purity. BCN-G was incorporated into potato-starch films (5–15% v/v) yielding homogeneous, mostly transparent films with strong UV-blocking (>99% in tested regions) and numerically lower water vapor permeability (from 1.36 to 1.24 g.mm/m².h.kPa for the 15% BCN sample). However, differences in some barrier metrics were not statistically significant. Overall, BCN derived from genipap byproducts appears promising as a biodegradable reinforcing agent for starch-based food packaging, supporting circular economy goals and SDG 12 (Responsible Consumption and Production).

Keywords Nanocellulose · Fermentation · Sustainability · Biomaterials · Waste valorization

Introduction

The expansion of the food industry to meet the demands of a growing global population has resulted in substantial volumes of organic waste in post-processing environments [1]. The juice production chain has been found to contribute significantly to organic waste generation, with the citrus sector alone responsible for approximately 25 million tons of waste on an annual basis [2]. The necessity of effective control of post-harvest losses is paramount, and a range of factors must be given full consideration. These include the ripening stage of the fruit [3], in view of the fact that the transportation chain for fresh produce is characterized by a high degree of fragility. Moreover, the physiological damage caused by diseases within the production chain can lead to losses of up to 30%, which increases the volume of waste by up to 80% of the fruit's total volume [4].

In this context, research efforts have increasingly focused on the valorization of these residues, investigating their properties and potential applications across diverse sectors. Recent studies have emphasized the potential of this waste as a raw material for various products, including pharmaceuticals [5], animal feed [6], bioplastics [7], bioenergy [8], and printable materials [9]. Furthermore, this waste can be utilized as a low-cost carbon source to produce bacterial cellulose nanofibers (BCN) [10], signifying a promising approach to valorize these agro-industrial by-products.

The ensuing essay is intended to furnish the reader with a comprehensive overview of the extant literature on the subject. The utilization of agro-industrial waste (AIW) in the development of high value-added products (by-products) has emerged as a pivotal area of interest for both the commercial and environmental sectors. This approach has been demonstrated to reduce waste disposal and promote the implementation of sustainable practices in industrial processes [4]. Furthermore, AIWs have been shown to be advantageous for BCN production, as many of these by-products have high concentrations of soluble sugars and compositions that favor greater cellulose development. Examples of such research include the utilization of acerola [11], mango [12], potato juice [13], and apple waste [14]. The present study focuses on genipap (*Genipa americana* L.) and soursop (*Annona muricata* L.). The genipap has a high soluble sugar content of 422.72 mg/g, of which around 170.83 mg/g is sucrose. In contrast, soursop is composed of 67% pulp and 33% kernel, seeds and skin [15]. Its soluble solids content is 151°Brix for the pulp and 1.51°Brix for the seeds, accounting for 93.6% of the total sugar content (fructose and glucose) [16]. In this instance, the by-product is rendered energetically viable for BCN production. The existing literature on this subject is somewhat superficial and lacks comprehensive comparative analysis with other AIWs. Research on BCN obtained from soursop wastes has been limited, despite its recent identification as a promising source for obtaining these biomaterials [17]. There is significant potential to explore the application of BCN in other polymer matrices, with the objective of developing advanced and sustainable composites for food packaging.

The AIWs utilized in this study are widely accepted in the Brazilian market, originating from tropical regions of the Brazilian Amazon, with unique characteristics that can impact the morphological and crystallographic variations of BCN [18, 19]. The by-products of these raw materials can function not only as sources of fermentable carbon (monosaccharide and disaccharide), but also as sources of bioactive and phenolic compounds that can confer functional properties to BCN (antioxidant, antimicrobial, or improved matrix/reinforcement interaction) [11–15, 17]. Consequently, research has committed to promoting circular values in the BCN production chain, with a special focus on sustainable packaging and materials.

Bacterial cellulose is characterized by its high purity and is composed of fibrils up to 100 nm in width. Chemically, it consists of β -D-glucopyranose units connected by β -1,4 glycosidic bonds. Its properties include high crystallinity, flexibility, and tensile strength [20, 21]. The production of bacterial cellulose can occur via an aerobic fermentation route (both static and dynamic), through extracellular and intracellular polymerization by bacteria and yeast, which utilize carbon sources such as glucose [22]. Among the known bacterial groups, *Komagataeibacter xylinus* is the most extensively studied, particularly under controlled temperature and humidity conditions [23].

Recently, microbial consortia such as SCOBY (Symbiotic Culture of Bacteria and Yeasts) have evolved from biological curiosities into key protagonists in bacterial cellulose production, owing to their metabolic versatility [24]. Within these ecosystems, cellulose synthesis by acetic acid bacteria is enhanced by yeasts, which play a strategic role in substrate hydrolysis and the provision of essential cofactors, such as vitamins and organic acids [25]. This cooperation not only optimizes carbon utilization but also enables the efficient conversion of agro-industrial residues into high-value biopolymers [26]. The ability of these consortia to thrive under less stringent sterile conditions makes them highly attractive for bacterial cellulose production that is both scalable and aligned with the principles of the circular bioeconomy [27].

Bacterial cellulose is a commercially valuable product with applications in a variety of fields due to its multifunctionality, versatility, and biocompatibility [17, 28, 29]. However, one of the challenges in producing this material is the high manufacturing cost, where more than half of the total expense is attributed to the cost of the culture medium, posing a significant barrier to industrial-scale production [10]. In this context, the development of BCN, using AIW as a culture medium, offers a viable (low-cost), environmentally friendly (sustainable) alternative with technological potential for packaging applications [10].

This study aimed to produce bacterial cellulose nanofibers (BCN) using new fermentable carbon (glucose, fructose, and sucrose) sources from agro-industrial waste from *Annona muricata* L. (soursop) and *Genipa americana* L. (genipap) originating from the Brazilian Amazon region. In addition to exploring the potential of waste as alternative sources for sustainable production, the BCN formed were characterized in terms of their morphological and crystallographic properties. These were then applied to flexible films for the study of food bio-packaging.

Materials and methods

Materials

Agro-industrial waste from soursop (*Annona muricata* L.) (AIW-S) and genipap (*Genipa americana* L.) (AIW-G), including peel, seeds, and portions of discarded pulp due physical damage, was used as an alternative source of fermentable carbon. This waste was obtained from COOPAAGRO – Cooperativa Agropecuária e de Serviços Nossa Senhora de Aparecida (Caucaia, BR) (3°48'59"S, 38°39'48"W). The starter culture, SCOBY (Symbiotic Culture of Bacteria and Yeasts), was provided by the Nutrition Laboratory of the Federal University of Ceará (Fortaleza, BR), its characteristics are described by da Silva et al. [26]. Low-density sodium carboxymethyl cellulose – CMC (Sigma-Aldrich, CAS 9004-32-4, D.S. 0.65–0.90) was also used.

Methods

Preparation of substrates for fermentation

Kombucha (BCN-control) was obtained from green tea (*Camellia sinensis* K.) leaves and stems purchased from Chá da Amazônia e Ervas (Amazônia, Brazil). The infusion was prepared at a concentration of 2.5 wt%, using water at 70 ± 5 °C for 15 min. The solution was then cooled to room temperature, and its pH was adjusted to 3.5–3.7 using a previously fermented liquid as starter culture, ensuring suitable acidic conditions for microbial growth and cellulose biosynthesis. Subsequently, 7.0 wt% of commercial refined sugar (99–99.8% sucrose, União, Brazil) was added, and 10 wt% of the starter culture (SCOBY) was inoculated into the medium. The value of 7.0 wt% corresponds to the formulation-based sucrose addition, whereas the initial sucrose concentration experimentally determined by HPLC is reported in Table 2 (mg/mL), representing the analytically measured concentration in the prepared medium. The AIW of soursop (AIW-S) and genipap (AIW-G), followed the same preparation procedure as the BCN-control, but without the incorporation of sucrose and green tea. The concentrations of fermentable soluble sugars (fructose, sucrose, and glucose) were recorded, maintaining a concentration of 7.0 wt% (Table 1). At the end of the soluble sugar extraction process, at room temperature, the extracts had their pH adjusted to 3.5–3.7 with the starter culture liquid.

Table 1 Fermentable carbon sources from raw materials that were considered for bacterial cellulose bio-membrane preparation by static fermentation

Raw Material	Fermentable carbon	Code
Commercial sugar ¹	Sucrose ³	BCN-control
Soursop ²	Sucrose ⁴ , fructose, and glucose	BCN-S
Genipap ²	Sucrose ⁴ , fructose, and glucose	BCN-G

(¹) Average granule size of sugar crystals between 10 and 20 µm; (²) Agro-industrial waste of soursop (*Annona muricata* L.) and genipap (*Genipa americana* L.): pulp, peel, seeds, and 30% fibrous part discarded due to industrial non-conformity; (³) This term is used to denote the sucrose content of commercial sugar; and (⁴) corresponds to the sucrose content of AIW-S and AIW-G. It is important to note that sucrose is not added during the fermentation process

Characterization of AIW and substrates

Fermentable sugars (sucrose, fructose, and glucose) were determined through high-performance liquid chromatography (HPLC) with SCL-10Avp controller system, SPD-10MAvp UV-VIS detector and LC-10Atvp gradient pump (Shimadzu, Kyoto, JPN) [30]. Calibration of the chromatograph employed external standards, including D(+) anhydrous glucose P.A. (Vetec, BR), sucrose P.A. (CAAL, BR), and D(-) fructose (Dinâmica, BR), prepared by dissolving 100 mg of the respective standard in 10 mL of Milli-Q water. Extracts were injected in triplicate into a liquid chromatograph. The analytical column was a Kromasil 100 Å, NH₂ (150 × 4.6 mm, packed with 5 µm particles). The mobile phase flow rate was 1.0 mL.min⁻¹ with a column temperature of 40 °C. The mobile phase comprised 87% acetonitrile (Carlo Erba, BR) and 13% Milli-Q grade water. The injection loop was set to 20 µL with a retention time of 20 min. The chromatographies of soursop and genipap extracts are presented in the supplementary material (Fig. S1). The results were based on the retention times of commercial standards (fructose, glucose, and sucrose). The quantification followed the methodology proposed by Lima et al. [30], whose recovery was greater than 95% and detection limits lower than 0.1 mg/mL.

Ground samples of AIW-S and AIW-G were used to prepare an extract at a 1:10 waste/water ratio, according to the method adapted from Assis et al. [31]. The extracts were centrifuged (15,303 xg) at 10 °C for 10 min (Heraeus Multifuge X3R—Thermo Scientific, Osterode Am, GER) and filtered in the qualitative filter. An aliquot of 1 mL of the material was diluted in 10 mL of the extraction solution (acetonitrile/water Milli-Q®) and filtered through a 0.22 µm filter. Quantification of fermentable sugars in the samples was expressed as mg per 100 g of waste. The same procedure was applied for quantifying sugars in the fermentation liquid at four different times: 0, 96, 192, and 360 h of fermentation. The total fermentable sugar (concentration was calculated by summing the concentrations of these three sugars (sucrose, glucose, and fructose) at each time point. The results were then presented in units of mg/mL.

The apparent product-to-substrate yield coefficient ($Y_{Productivity/Sugar,apparent}$) was calculated based on the net consumption of fermentable sugars, quantified by HPLC as the sum of sucrose (S), glucose (G), and fructose (F), between the initial fermentation time (t_0) and the final time point (360 h). The yield was defined as the ratio between the dry mass of bacterial cellulose produced (BC yield, g/L) and the net decrease in total measured sugars ($\Delta(S + G + F)$, mg/mL), according to Eq. 1.

$$Y_{Productivity/Sugar,apparent} = \frac{BC \text{ Yield}}{(S + G + F)_{(t_0)} - (S + G + F)_{(t_{360})}} \quad (1)$$

Production, purification, neutralization, and suspension of BC

The SCOBYs formed at the air-water interface of the extracts (control, AIW-S, and AIW-G) were removed and carefully washed with distilled water at 80 °C/1 h (three repetitions) to eliminate residues from the fermentation process (Fig. 1B). Subsequently, the samples were purified in 2% NaOH solution (w/v) at 80 °C/1 h (two

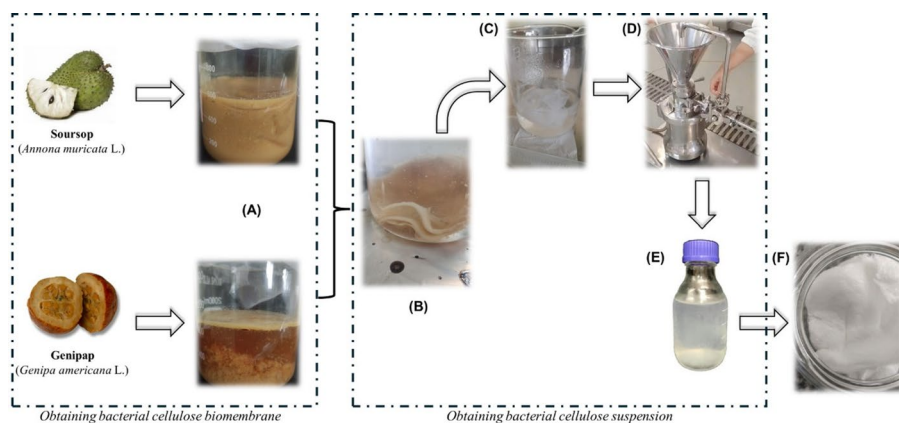


Fig. 1 Process for obtaining bacterial cellulose suspension from AIW-S and AIW-G: **(A)** waste extracts with pH adjustment to 3.5–3.7; **(B)** purification in distilled water (80 °C for 60 min); **(C)** neutralization in 2% NaOH (80 °C for 120 min), followed by washing with distilled water until reaching a neutral pH; **(D)** use of a colloidal mill, with bacterial cellulose suspension stabilized with 0.1% CMC (w/w); **(E)** bacterial cellulose suspension at 2% (m/v); and **(F)** fibrous mat of freeze-dried bacterial cellulose

repetitions) (Fig. 1C). At this stage, cellulose bleaching occurs to remove the dark coloration derived from the carbon source. After purification, the bacterial cellulose (BC) membrane was neutralized by washing with distilled water at 80 °C until a pH close to 7.0 was reached [32].

The bacterial cellulose suspension (Fig. 1E) was prepared following the methodology proposed by Andrade et al. [33], with adaptations. Defibrillation of the neutralized BC membrane was conducted in stages (Fig. 1D): (i) increasing the membrane's surface area through cutting and grinding; (ii) obtaining a concentrated BC suspension at 2% (w/v) with 0.1% (w/w) carboxymethylcellulose (CMC) as a stabilizing agent, using a colloidal mill (Meteor Rex Inox I-V-N) from São Paulo, BR with recirculation for 10 min. The suspension was stored under refrigeration for later use in the characterization of the obtained bacterial cellulose nanofibers. The cellulose suspension was frozen in an ultrafreezer model MDF-U33 V (SANYO, Moriguchi, JPN) at -80 °C and freeze-dried (1,000–2,000 µmHg) in a pilot freeze-dryer model LP 510 (Liobrás, São Paulo, BR) for 48 h, initial of the -100 to -30 °C, increasing up to 30 °C (Fig. 1F) [34].

The total BC yield and the productivity of the biosynthesis of the different substrates under fermentation were calculated according to the method proposed by Volova et al. [35] (Eq. 2 e 3).

$$Y = \frac{m_{BC}}{V_{cultive}} \quad (2)$$

$$P = \frac{Y}{t} \quad (3)$$

Where Y corresponds to the yield (g.L^{-1}); m_{BC} , the dry mass of cellulose (g); V, the volume of the culture medium used (L). Therefore, productivity (P) will be the ratio of the yield divided by the time (t) in days. The experiment was performed in duplicate.

Characterization of bacterial cellulose nanofibers

The morphologies of the cellulose nanofibers were analyzed using a Quanta FEG 450 microscope (FEI Company, US). Samples were mounted on aluminum stubs using double-sided carbon adhesive tape and then coated with a 20 nm layer of gold at an acceleration voltage of 20 kV [34]. The mean fiber diameters were calculated using ImageJ software (National Institutes of Health, Bethesda, MD, US), analyzing the morphology of 55 randomly selected fibers.

Fourier transform infrared (FTIR) spectroscopy was performed using a PerkinElmer spectrophotometer (Spectrum Two LiTa, Liantrisant, UK) equipped with an attenuated total reflectance (ATR) sampling accessory. The analysis was conducted in the range of $4,000$ to 500 cm^{-1} , with a resolution of 4 cm^{-1} over 64 scans. The spectra were obtained in transmittance (%) mode, and the characteristic absorption peaks were identified and discussed qualitatively.

X-ray diffraction (XRD) analysis was conducted using an Ultima IV diffractometer (Rigaku, JPN). The crystallographic patterns of the dried samples (Fig. 1F) were examined using Cu-K α radiation ($\lambda = 1.54056 \text{ \AA}$), operating at 40 kV and 20 mA, with a scanning rate of $5^\circ/\text{min}$ and a step size of 0.02 s^{-1} . The samples were prepared according to Fig. 1. Thus, the lyophilized samples (Fig. 1F) were evaluated over a 2θ range from 5° to 44° . The crystallinity index was calculated using DIFFRACT. EVA software (Bruker) based on the total area under the diffractograms between 5° and 44° [17]. The interplanar spacing (d-spacing), as per Bragg's Law (Eq. 4), and the cellulose crystallite size were determined using the Scherrer method (Eq. 5), as proposed by Pal et al. [36].

$$d = \frac{n\lambda}{2\sin(\theta)} \quad (4)$$

$$\langle D_{av} \rangle = \frac{k\lambda}{\beta \cos(\theta)} \quad (5)$$

where $\langle D_{av} \rangle$ is the average crystallite size; n is reflection order (considered for the main peak); k is the shape factor (0.9), λ is the X-ray wavelength ($1.54056 \text{ \AA}$), θ is the Bragg angle corresponding to the diffraction plane (002), and Full Width at Half Maximum (FWHM) (β) converted to radians.

Thermogravimetric analysis (TGA) was performed to study the thermal degradation (i.e. mass loss) of the samples. The analysis was conducted using a Shimadzu DTG-60 H (Kyoto, JPN) set to a heating ramp of $10^\circ\text{C}/\text{min}$ in a nitrogen atmosphere with a constant gas flow of $50 \text{ mL}/\text{min}$, heating from 23°C to 800°C . T_{onset} determination (initial degradation temperature) indicates the temperature at which the sample begins to degrade. The endpoint of the thermogravimetric (TG) curve

indicates the percentage mass loss. Furthermore, peaks in the DTGA curves, derived from the TG analysis, indicate the temperatures at which the highest rates of thermal degradation occur [37].

Differential Scanning Calorimetry (DSC) analysis was conducted using a Q20 V24.9 Build 121 (TA Instruments, New Castle, GER) under an N₂ atmosphere with a constant gas flow of 50 mL/min. The temperature range applied was from −50 °C to 210 °C, with a heating rate of 10 °C/min, aiming to determine the glass transition temperature (T_g) and melting temperature (T_m) during the heating scan.

Evaluation of BCN-G as a composite agent in flexible films

To study the impact of BCN on the optical and moisture barrier properties of flexible films, commercial Brazilian potato starch was used as a model biopolymer. The film-forming solution contained 6.0 wt% potato-starch, 0.25 g/g glycerol (a biopolymer plasticizer) and various concentrations of BCN-G (5%, 10%, and 15% v/v). All polymer solutions were prepared with distilled water and heated to 80 °C for 5 min. The solutions were then homogenized in an ultrasonic mixer (model T18, IKA®, BR) at 15,500 rpm for 3 min and degassed in a centrifuge (Hettich®, model 380R, GER) at 10,000 rpm for 5 min. The films were obtained using the casting method with a wet thickness of 1.0 mm. The films were left overnight under ambient conditions of temperature (25 ± 2 °C) and relative humidity (65 ± 5%). The thickness of the dry films was measured using a digital micrometer (accuracy of 0.001 mm, Fugisan, CHN).

Water vapor permeability (WVP) and water vapor transmission rate (WVTR) analyses were performed according to ASTM E96/E96M-16 [38], at a temperature of 25 ± 2 °C. For the desiccant method, silica gel (30% RH) was used in a vertical Dry-Box desiccator (Hipperquimica, SP, Brazil). Permeation cells (Ø 24 mm and 10 mm high) with 2.0 mL of distilled water (100% RH) were used. Eight measurements were performed in 24 h. The data obtained were calculated according to Eq. 6.

$$WVP = \frac{g \times e}{A \times t \times \Delta P} \quad (6)$$

Being WVP (g.mm/m².h.kPa) the permeability; g, the mass gain (g); e, the average thickness (mm); A, the permeation area (m²); t, total time (h); ΔP, the ambient vapor pressure difference (kPa, 25 °C); and g/t calculated by linear regression of weight gain versus time curve (permanent regime). The WVTR (g/m².h) corresponds to the

relation $\frac{g}{A \times t}$. Five repetitions were performed for each sample.

For the optical properties of the films, the methodology proposed by Haghighi et al. [39] was used for transparency and opacity. Film samples (1 cm x 5 cm) were placed in a glass cuvette, oriented perpendicular to the light beam of a UV spectrophotometer (model UV-1800, Shimadzu, JPN). Transparency was determined based on the transmittance at 600 nm (Eq. 7).

$$Transparency = \frac{-(\log \%T_{600})}{b} \quad (7)$$

Where % T_{600} represents the percentual transmittance at a wavelength of 600 nm and b is the thickness of the film in millimeters (mm).

Regarding the samples' opacity, Eq. 8 was first used to calculate the absorbance at 500 nm [40], then opacity was determined according to Eq. 9:

$$Abs_{500} = -\log \frac{1}{T} \quad (8)$$

$$Opacity = Abs_{500} \times b \quad (9)$$

Where T is the transmittance value at 500 nm, Abs_{500} is the absorbance acquired at 500 nm, and b is the film's thickness (mm).

UV-shielding percentages were performed from 200 to 400 nm, aiming to evaluate the barrier in three different wavelength UV regions: S_{UVC} (200–280 nm), S_{UVB} (280–320 nm), and S_{UVA} (320–400 nm). The results were determined according to Eqs. 10–12 [41]:

$$S_{UVA} = 1 - T_{(UV-A)} = 1 - \frac{\sum_{320}^{400} T_{\lambda} \times \Delta\lambda}{\sum_{320}^{400} \Delta\lambda} \times 100\% \quad (10)$$

$$S_{UVB} = 1 - T_{(UV-B)} = 1 - \frac{\sum_{280}^{320} T_{\lambda} \times \Delta\lambda}{\sum_{280}^{320} \Delta\lambda} \times 100\% \quad (11)$$

$$S_{UVC} = 1 - T_{(UV-C)} = 1 - \frac{\sum_{200}^{280} T_{\lambda} \times \Delta\lambda}{\sum_{200}^{280} \Delta\lambda} \times 100\% \quad (12)$$

Where, T_{UV-A} , T_{UV-B} , and T_{UV-C} are the averaged transmittance (%) in specified UV-regions, λ and $\Delta\lambda$, wavelength and wavelength spread, respectively, and T_{λ} is the spectral transmittance.

Statistical analysis

Statistical analysis was carried out using Statistica 12 (StatSoft, Inc., US, 2011). One-way ANOVA was applied to detect significant effects, and Tukey's HSD test was used for pairwise comparisons, with significance defined at $p < 0.05$.

Results and discussions

Quantification of fermentable sugars by HPLC

The total sugar concentration in the fresh residues was found to be elevated. For AIW-S, the total was 24.90 mg/100 g fw, with fermentable sugars composed of fructose (10.72 ± 0.35 mg/100 g fw), glucose (13.08 ± 0.29 mg/100 g fw), and sucrose

(1.11 ± 0.00 mg/100 g fw). It was determined that AIW-G was the most abundant sample, with a total of 41.99 mg/100 g fw, comprising fructose (8.88 ± 0.15 mg/100 g fw), glucose (16.91 ± 0.39 mg/100 g fw) and sucrose (16.20 ± 0.06 mg/100 g fw). However, when preparing the extracts by the infusion method, the highest concentrations of fermentable carbon at the initial moment (t_0) were observed over 360 h (15 days), as shown in Table 2.

As presented in Table 2, the three substrates: control, AIW-S, and AIW-G exhibit different carbohydrate availability and transformation profiles, which are reflected in the initial substrate composition and the functional activity of the symbiotic consortium of microorganisms. In BC control, glucose and fructose concentrations were not detected until 96 h, which can be attributed to the activity of invertase derived from yeasts present in the symbiotic consortium. Studies demonstrate this action of the symbiotic consortium facilitated by the action of yeast invertases [42, 43]. Accordingly, the low (G+F)/S ratio at the initial stage (0.07) suggests that there is minimal measurable monosaccharide release before fermentation begins.

In contrast, the genipap-based substrate (AIW-G) exhibited the highest total fermentable sugar content (661.65 mg/mL) and a markedly elevated (G+F)/S ratio (3.56), indicating a high degree of sucrose hydrolysis during fermentation. The temporal profiles of individual sugars revealed substantial monosaccharide availability: glucose concentrations increased between 120 and 192 h, then declined gradually, while fructose levels remained comparatively high before declining later. These patterns are indicative of dynamic sugar transformation and utilization over time, associated with the progressive decline in sucrose concentration. It is imperative to interpret these dynamics as system-level behavior resulting from concurrent hydrolysis and consumption processes within the symbiotic consortium, rather than as evidence of preferential metabolic uptake.

Soursop (AIW-S) presented an intermediate total sugar content of 438.73 mg/mL and a (G+F)/S ratio of 1.99, suggesting a considerable degree of sucrose hydrolysis during the fermentation process. The sugar concentration profiles reported in Table 2 indicate greater apparent glucose utilization than fructose at the system level, consistent with glucose as the preferred carbon source under the studied conditions. These patterns are indicative of the combined effects of sucrose hydrolysis and subsequent consumption of the resulting monosaccharides by the symbiotic microbial consortium. The dynamics of sugar consumption may be further influenced by the composition of the symbiotic culture and the interactions among its microbial members, as reported in previous studies [43–46].

At the final fermentation stage (360 h), a significant decrease in total fermentable sugars was observed across all treatments, suggesting substantial microbial utilization of carbohydrates. This decline is consistent with the conversion of sugars into organic acids, ethanol, microbial biomass, and bacterial cellulose, as is commonly reported for kombucha fermentation systems [47]. Overall, the temporal sugar profiles highlight extensive carbohydrate utilization derived from sucrose hydrolysis and dynamic monosaccharide availability during fermentation. While the concentration patterns indicate glucose as the preferred carbon source at the system level under the studied conditions, the data do not allow for the determination of a specific metabolic uptake mechanism by the kombucha microbiota.

Table 2 The evolution of the concentration of fermentable sugars (mg/mL) (sucrose – S, glucose – G, and fructose – F) over a period of 360 h of static aerobic fermentation with SCOBY in different agro-industrial wastes is presented: commercial sugar (control), soursoy (AIW-S), and genipap (AIW-G)

Time (h)	Control			AIW-S			AIW-G		
	S	G	F	S	G	F	S	G	F
0	84.47±0.06	< LOQ*	< LOQ	29.80±0.03	28.03±0.08	15.43±0.01	27.64±0.04	50.66±0.00	31.63±0.01
96	77.46±0.01	< LOQ	< LOQ	23.68±0.09	28.92±0.03	17.36±0.02	27.71±0.02	50.19±0.02	22.86±0.01
120	59.27±0.02	2.03±0.02	2.92±0.07	28.02±0.03	27.85±0.05	16.73±0.07	19.11±0.07	69.30±0.01	32.69±0.02
192	63.16±0.09	2.35±0.07	3.80±0.03	33.74±0.05	36.06±0.05	18.45±0.03	18.24±0.01	64.34±0.09	37.30±0.00
264	74.32±0.01	3.43±0.07	5.45±0.09	20.23±0.09	50.08±0.03	12.33±0.06	41.33±0.07	58.64±0.02	32.61±0.01
360	10.68±0.02	2.94±0.01	4.21±0.07	11.13±0.03	31.80±0.04	9.09±0.01	11.22±0.02	40.06±0.07	26.12±0.05
$\sum \text{sugars}$ (mg/mL)	396.49±0.63			438.73±0.78			661.65±0.72		
$(G + F/S)$	0.07			1.99			3.56		
$\gamma_{P/S}^{\text{ratio}}$ (g/g) [†]	0.47			0.45			0.45		

(*) below the limit of quantification; (†) Values represent the cumulative sum of sucrose, glucose, and fructose concentrations measured at 0, 96, 192, and 360 h, serving as an indicator of total carbohydrate availability

The calculated apparent yield coefficients ($Y_{P/S,app}$) for AIW-S and AIW-G were 0.45 g/g, suggesting that the conversion of consumed sugars into bacterial cellulose was maintained when Amazonian fruit substrates were used.

Yield and productivity of BC

Cellulose yield and productivity depend on the composition of the substrate used for fermentation, as well as the type of fermentation method (static or dynamic), the area of the medium, the volume ratio, and the pH used [22, 35]. Table 3 shows the yield and productivity of bacterial cellulose after 360 h of static aerobic fermentation.

Table 3 shows the control treatment exhibited higher yield (31.05 g/L) and productivity (1.77 g/L.day) in comparison to the residues. Therefore, the sequence is as follows: $Y_{control} > Y_{genipap} > Y_{soursop}$, and consequently, productivity is $P_{control} > P_{genipap} > P_{soursop}$. Consequently, this discrepancy in residues, with respect to the control, may be associated with the predominance of sucrose (Table 2) during the fermentation period, accompanied by a gradual and sustained release of monosaccharides to the symbiotic consortium of microorganisms. However, the high initial availability of monosaccharides (as in AIW-G) does not invariably result in high BCN yields. It can thus be concluded that the timing of the release and the chemical composition of the medium (including inhibitory or modulatory compounds) can be of fundamental importance in the production of cellulosic material [35, 48].

Characterization of bacterial cellulose

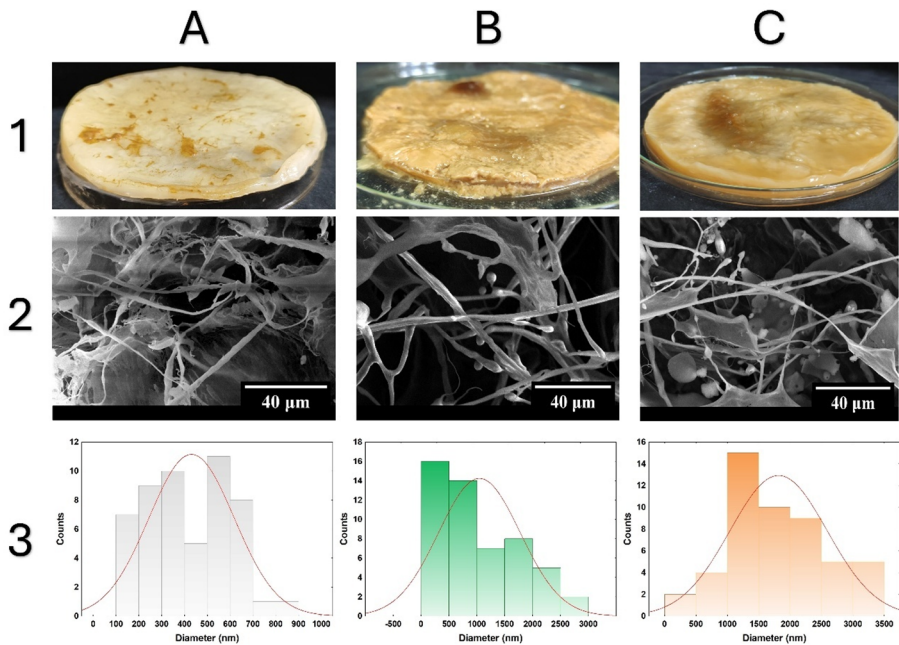
Changes in the fermentable carbon source and cultivation method can induce modifications in the three-dimensional morphology of the cellulose film, as well as in the size and shape of the fibers [49, 50]. Indeed, the BCN derived from alternative carbon sources exhibited morphological and structural variations in the fibril network (Fig. 2).

Figure 2A (line 1) shows macroscopic images of the SCOBY formed using the conventional static fermentation method. Figure 2A1 shows the SCOBY formed using *Camellia sinensis* K. and sugar (sucrose). Figure 2B1 and 2B3 show the SCOBYs formed using the alternative carbon sources of soursop (*Annona muricata* L.) agro-industrial waste (AIW-S) and genipap (*Genipa americana* L.) agro-industrial waste, respectively. The color difference in the SCOBYs can be observed and may be associated with the influence of the culture medium in terms of pigmentation, phenolic compound residues and/or protein content. This behavior has already been observed in similar studies with soybean whey, which promoted yellow pigmentation [51].

Variations in the nanofibrous network can also be observed in the micrographs (Fig. 2, line 2), with differences in organization and density that may indicate varying degrees of crystallinity. The diameter distribution of the nanofibers (Fig. 2, line 3) shows more uniform fibers (Fig. 2A3) and more random fibers (Fig. 2B3 and Fig. 2C3 with the formation of microfibrils ($>1000 \mu\text{m}$) and nanofibers ($<500 \text{ nm}$) evident in the micrographs. However, it is challenging to estimate the diameter/size of cellulose fibers due to their fibrillated form [52]. Furthermore, microparticles

Table 3 Data on bacterial cellulose yield and productivity after 360 h (15 days) of static fermentation under ambient conditions (temperature, 25 °C, and relative humidity, 50–65%)

	Control	BCN-S	BCN-G
Yield (g/L)	31.05 ± 0.64	9.56 ± 0.13	14.50 ± 0.60
Productivity (g/L.day)	1.77 ± 0.38	0.63 ± 0.01	0.96 ± 0.03

Means ± standard deviation ($n=2$)**Fig. 2** SCOBY of BC *in natura* (unwashed and unpurified) cultivated in *Camellia sinensis* tea (green tea) + sucrose (A1), soursoy AIW (B1), and genipap AIW (C1), followed by micrographs of the nanofibers networks (purified BCN) (A2), (B2), and (C2), and diameter histograms ($n=55$) (A3), (B3), and (C3) for each respective fermentable carbon sources

attached to the nanofibers can be observed, which may indicate an improvement in the bleaching and/or neutralization protocols of the biomembrane.

Ioelovich and Leykin [53] propose that the average diameter of bacterial cellulose fibrils is approximately 100 nm, with a length of up to 100 μm. However, other studies have shown that the diameter of bacterial cellulose fibrils can vary over a much wider range (typically from 20 to 500 nm), depending on the microbial strain or consortium, the carbon source, the fermentation conditions, and the post-treatment processes [17, 20, 54]. Additionally, the literature suggests that the morphology and chemical composition of the cellulose surface can be influenced by the carbon source materials, as well as by the treatment processes used to obtain the biomaterial, including factors such as microbial strain type, temperature, incubation conditions, and incubation time [4, 49, 55–57]. Vinogradov et al. [52] propose that the formation and structuring of these fibrils arise from the process by which microorganisms (bacteria

and/or yeasts) synthesize these long macromolecules. The authors also highlight the challenges in controlling or predicting potential fibril entanglements.

FTIR analysis was conducted to investigate the vibrational frequencies of the materials, providing insights into the material's molecular structure and physico-chemical properties [58]. The infrared (IR) spectra of bacterial nanocellulose (BCN) obtained from different fermentable carbon sources are presented in Fig. 3. The results revealed that, although the characteristic absorption peaks indicated that bacterial cellulose remained consistent among the samples, variations were observed among the spectra (Fig. 3). Table 4 presented the main peaks identified and their respective assignments.

Analysis of the IR spectra revealed that the stretching peak at approximately 3345 cm^{-1} , attributed to O–H stretching vibrations associated of type I cellulose, showed no significant variation between different carbon sources (Fig. 4). This behavior is consistent with the importance of hydrogen bonds in maintaining the structure and organization of cellulose [70]. Previous studies have shown that the purification process using an alkaline solution containing NaOH can lead to changes in the OH stretching vibrations [61]. Furthermore, this peak may be associated with the cellulose purification step due to the reduced intensity of the $\nu(\text{NH})$ stretching peak (Gea et al., 2011). However, no component was incorporated during the fermentation process for the visualization of the 1730 cm^{-1} peak corresponds to C=O stretching in proteins and lipids [62, 63], which indicates a possible failure in the purification protocol and suggests the presence of residual organic compounds derived from the substrate and/or fermentation by-products, possibly due to incomplete removal during the alkaline purification step. Similar observations have been reported in the lit-

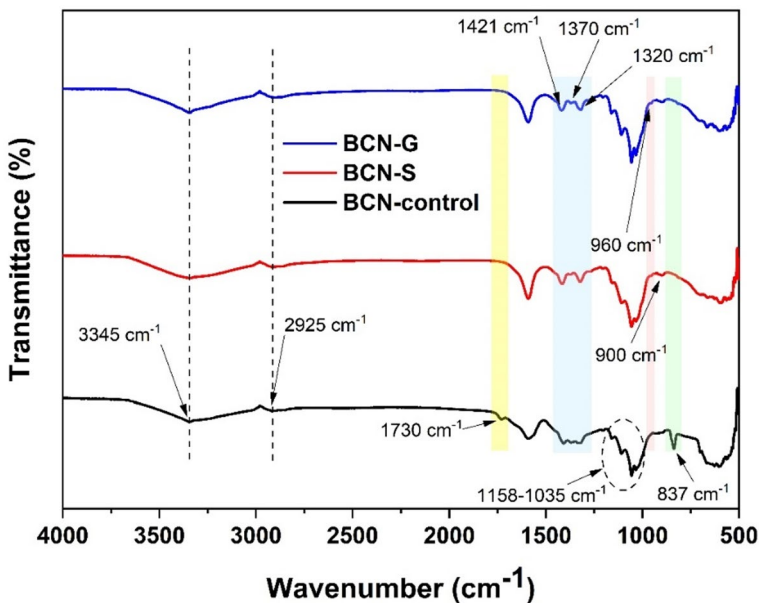
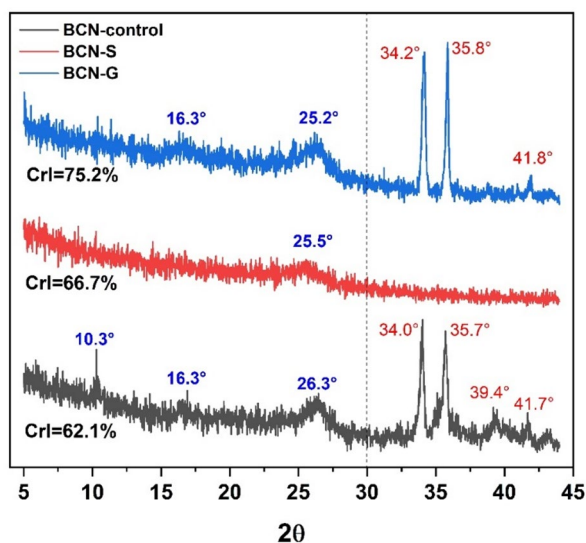


Fig. 3 ATR-FTIR spectra (in transmittance mode) of the bacterial cellulose nanofibers (BCN) films obtained from kombucha (control) and soursop (S) and genipap (G) agro-industrial wastes

Table 4 Main peaks and their respective functional groups identified and assigned for bacterial cellulose (BC) obtained from different fermentable carbon sources

Wavenumber (cm ⁻¹)	Functional groups	References
3345	$\nu(\text{O-H})$ cellulose I	[59, 60]
2925	$\nu_a(\text{CH}_2)$	[61, 62]
1730	$\nu(\text{C=O})$ form proteins and lipids	[62, 63]
1421	$\delta(\text{HCH, OCH})$ in the plane	[61]
1370	$\omega(\text{CH})$	[61]
1320	Benzene ring mixed with $\delta(\text{CH})$ in the plane (cellulose)	[62]
1158	$\nu_a(\text{C-O-C})$, CH deformation	[63, 64]
1111	Ring $\nu(\text{C-C})$ for polysaccharides, cellulose	[62]
1063	$\nu(\text{C-O})$	[64]
1035	$\delta(\text{C-O})$	[65]
900	Out-of-plane $\delta(\text{CH})$ vibrations	[66]
960	ν (β -glycosidic bond) out-of-plane	[67]
837	$\nu(\text{C-O-C})$ with β -1,4 linkage at the anomeric carbon	[68, 69]

ν (stretching); ν_a (asymmetric stretching); δ (bending)

Fig. 4 X-ray diffraction of BCN obtained from different fermentable carbon sources: BCN-control (sucrose), BCN-S (AIW- soursop), and BCN-G (AIW-genipap)

erature, indicating that insufficient purification protocols may lead to the persistence of ester and carbonyl-containing impurities [71]. In the infrared spectra of BCN-S and BCN-G, the 1730 cm⁻¹ peak is not evident. The band at 960 cm⁻¹ corresponds to out-of-phase stretching of the β -glycosidic bonds between the structural glucose units of bacterial cellulose [72] and is therefore related to the polysaccharide structure of bacterial cellulose (Fig. 2B–C); and 900 cm⁻¹, which is attributed to β -glycosidic bonds out of the ring plane. Additionally, the peak at 1160 cm⁻¹ is associated with the presence of OH groups of phenolic components and asymmetric stretching of

C–O–H bonds in 1,4- β -glycosides. At 1035 cm^{-1} , the peak corresponds to asymmetric stretching associated with cellulose crystallinity [67–69, 73, 74].

This study revealed that the symbiotic consortium used in the fermentation process involving alternative carbon sources produced primarily type I cellulose (3345 cm^{-1} , 1160 cm^{-1} and 900 cm^{-1}), with only trace amounts of type II cellulose (1421 cm^{-1} and 1320 cm^{-1}) detected. This pattern of behavior was like that observed in the BC culture using *Komagataeibacter* sp. W1 [65, 75]. Subtle variations in the intensities of the IR spectra, as well as the presence of secondary peaks, suggest that although the fundamental chemical structure of BC is maintained, the different compositions of the fermentation substrates may have influenced the molecular characteristics of the BCN network, as can be seen in Fig. 2.

The X-ray diffraction patterns of BCN obtained from different carbon sources are presented in Fig. 4. The diffractograms revealed significant differences among the samples, allowing a comparative evaluation of the crystalline structure.

XRD analysis revealed diffraction patterns with predominant peaks at 2θ angles of 16° , 25° – 26° , 34° – 35° and 41° – 42° (Fig. 4). Typical diffraction peaks of cellulose I reported in the literature are located at approximately 14.9° , 16.5° , 22.6° – 22.9° and 34° – 35° , with the most intense peak usually corresponding to the (002) plane near 22° [76–78]. In the present study, the BCN-control and BCN-G samples exhibited intense diffraction peaks around 34° – 35° , while the BCN-S sample showed a weaker diffraction near 25.5° . Therefore, although diffraction features compatible with cellulose I can be identified, the peak positions and relative intensities indicate deviations from an ideal cellulose I pattern, possibly due to structural disorder, coexistence of different cellulose polymorphs and/or the influence of non-cellulosic components derived from the fermentation substrates. The diffraction pattern observed in this study is consistent with the findings reported in the extant literature concerning bacterial celluloses. Zhu et al. [76], evaluated the biosynthesis of native bacterial cellulose and observed diffraction peaks at 14.4° , 16.6° , 22.6° , 27.8° and 29.3° , thus providing a valuable addition to the existing body of knowledge in this field. As French [77] demonstrated, models have been presented which associate the crystallinity of cellulose I with peaks near 16.3° , 22.5° and 34° . As demonstrated in Fig. 1, the peaks under consideration were found to be associated with the following diffraction planes: (101), (111), (002), (130) and (122), respectively. In a similar manner, when studying the synthesis of bacterial cellulose by *Komagataeibacter xylinus* with aqueous orange peel extract, Padmanabhan et al. [79] observed the predominance of the diffraction planes (101), (111) and (002), which correspond to the 2θ peaks at 14.4° , 16.6° and 22.6° , respectively.

Furthermore, Fig. 4 demonstrates the presence of diffraction peaks that exceed 30° , specifically between 34° and 35° and between 41° and 42° , which may be associated with the following: The factors to be considered are as follows: (i) impurities; (ii) degradation products; (iii) structural rearrangements resulting from the heat treatment and pH applied to the fermentation extracts; and (iv) interactions and complexation with bioactive phenolic compounds and/or minerals, as well as the crystallographic organization of bacterial cellulose [80–83]. Evidence of these possible impurities was also observed in the micrographs (Fig. 2) and IR spectra (Fig. 3).

The variations observed in the diffraction peaks suggest that the carbon source used in the fermentation process may influence the structural organization and the degree of crystallinity of the resulting bacterial nanocellulose (Table 5).

The crystallinity index (CrI) exhibited divergent values in relation to the substrates: control (CrI=62.1%), BCN-S (CrI=66.7%), and BCN-G (CrI=75.2%). The findings indicate that utilizing AIW as a carbon source promoted the molecular organization and compaction of the crystal lattice, particularly in the AIW-G substrate. However, according to French [84], minor variations in diffraction and crystallinity angles may be indicative of changes in the structural organization of cellulose, potentially associated with carbon sources and variations in process conditions. In the present study, it was observed that the substrates derived from agro-industrial wastes (soursop and genipap) resulted in higher crystallinity indices than the control (Table 5), which may be related to differences in sugar composition and bioavailability in the culture media derived from these substrates, rather than solely to the predominance of specific monosaccharides. Consequently, variations in the chemical composition of the culture media may have promoted differences in cellulose biosynthesis and nanofiber packing, thereby facilitating an increase in the crystalline fraction, which is consistent with the observations presented in Tables 2 and 5.

The relative peak intensities of the samples demonstrated significant variations, with BCN-G exhibited higher crystallinity and notably narrower peaks (FWHM=0.4992). In contrast, the broader peaks observed in BCN-control and BCN-S (FWHM=0.9984) may indicate structural heterogeneity, potentially stemming from a lower crystalline fraction and the presence of defects or amorphous regions [85, 86].

Therefore, BCN-control and BCN-S demonstrated similar crystallite sizes of approximately 82 nm. In contrast, BCN-G presented a larger crystallite size, approximately 163 nm. However, although the average crystallite sizes vary, BCN-control and BCN-S had smaller crystallites (81.7 and 81.6 nm, respectively) and interplanar spacing of 0.339 nm and 0.349 nm, respectively. For BCN-S, the increase in interplanar spacing, relative to BCN-control, can be attributed to the presence of impurities in the substrate, which may have caused variations in the crystal lattice [87].

Ciolacu and Popa [81] established a hierarchical classification system for CrI cellulose polymorphs, wherein $\text{CrI}_{\text{cellulose I}} > \text{CrI}_{\text{cellulose II}} > \text{CrI}_{\text{cellulose III}}$. The BCN-control sample (Fig. 4) exhibited a diffraction peak at 10.3° , which may be associated with: (i) the presence of cellulose II or cellulose III [81] due to partial structural rearrangements of cellulose chains during processing and purification steps and (ii)

Table 5 Physical parameters determined by X-ray diffraction (XRD) analysis of the BCN samples are as follows

Samples	CrI (%) ¹	2 Θ (°)	FWHM ²	< D _{av} > (nm) ³	d-spacing (nm) ⁴
BCN-control	62.1	26.3	0.9984	81.7	0.339
BCN-S	66.7	25.5	0.9984	81.6	0.349
BCN-G	75.2	25.2	0.4992	163.1	0.353

(¹) Crystallinity index; (²) FWHM (Full width at half maximum) of the XRD diffraction peak; (³) Average crystallite size using the Scherrer equation; and (⁴) Calculated using Bragg's law: $n\lambda = 2d\sin(\Theta)$

Fig. 5 Thermal weight analysis curve of BCN – control, sour-sop AIW (BCN-S), and genipap AIW (BCN-G)

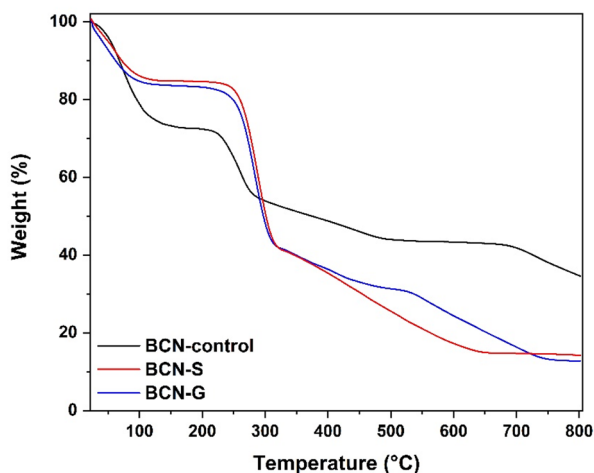


Table 6 Thermogravimetric analysis of BCN control samples and BCN obtained from agro-industrial waste of soursop (BCN-S) and genipap (BCN-G)

Sample	1st thermal degradation		2nd thermal degradation		3rd thermal degradation		Residual
	$T_{\text{On-EndSet}}$ (°C)	^1LW (%)	T_{OnSet} (°C)	LW (%)	T_{OnSet} (°C)	LW (%)	
BCN-control	23–162	27	226	30	685	8	35
BCN-S	23–139	15	260	70	*	*	15
BCN-G	23–139	16	273	53	538	18	13

(¹) corresponds to weight loss represented as a percentage; (²) residual percentage and (³) residual mass concerning the initial mass used in the TGA analysis; (*) Unidentified event

disordered (amorphous) cellulose phases due to interactions and complexation with bioactive (phenolic) compounds and/or minerals [80, 82].

Thermal analysis of the BCNs revealed up to three stages of degradation (see Fig. 5). The BCNs exhibited different behaviors during the initial stage of mass loss, which corresponds to the evaporation of free (residual) water (Table 6). The temperature range for this stage was 23–162 °C for BCN-control and 23–139 °C for BCN-S and BCN-G. This behavior may be associated with the water retention capacity of BCN. However, variations in the thermal range of water evaporation are due to the strong hydrophilic interaction with hydroxyl groups (Table 4) and the crystallinity index (CrI) (Table 5).

In the second stage (see Table 6), an increase in the maximum thermal degradation temperature of BCNs can be observed. According to the literature, the decomposition of the main cellulose chain occurs between 260 and 380 °C [88, 89]. This process involves the depolymerization and/or breakdown of glucose units and the amorphous fraction [37, 51]. Thus, differences in the CrI of BCNs and in their compositional characteristics, including residual non-cellulosic components, may contribute to the observed thermal behavior, corroborating the observations presented in Table 5. The third stage is associated with the oxidation and decomposition of carbon-containing residues [51]. T_{onset} of BCN-G is higher than that of BCN-S and BCN-control, but

lower than that reported in the literature ($>335\text{ }^{\circ}\text{C}$) [51, 90]. The residues formed at $800\text{ }^{\circ}\text{C}$ correspond to the formation of carbonaceous materials. However, the high percentage for BCN-control can be attributed to impurities from the purification process.

Figure 6 illustrates the thermal behavior of the BCN obtained from different carbon sources obtained by differential scanning calorimetry (DSC) and thermal degradation (mass loss), respectively. No glass transition temperatures (T_g) were identified; however, a slight inflection was observed in the curves between 60 and $90\text{ }^{\circ}\text{C}$ (Fig. 6 *inset*). The temperature applied during the analysis may have induced polymer chain relaxation, resulting in an inflection in the DSC curve without necessarily causing a phase change [83].

The analysis of the DSC thermograms (Fig. 6) reveals an endothermic peak characteristic of organic compounds, such as cellulose, corresponding to the crystalline melting temperature (T_m). The thermograms display narrow peaks with significant amplitudes. The analysis of the DSC thermograms (Fig. 6) reveals that fusion enthalpy (ΔH_m) exhibits a decreasing trend: BCN-control (529.2 J/g) $>$ BCN-G (280.4 J/g) $>$ BCN-S (260.4 J/g). This thermal behavior suggests that while BCN-control possesses a lower crystallinity index (CrI) of 62.1% (Table 5), its crystalline domains likely possess a higher degree of perfection and purity, thus demanding more energy for their fusion. Conversely, BCN-G, despite exhibiting a significantly higher CrI of 75.2% (Table 5), shows a comparatively lower ΔH_m . This phenomenon may be attributed to the presence of impurities such as residual protein material, cul-

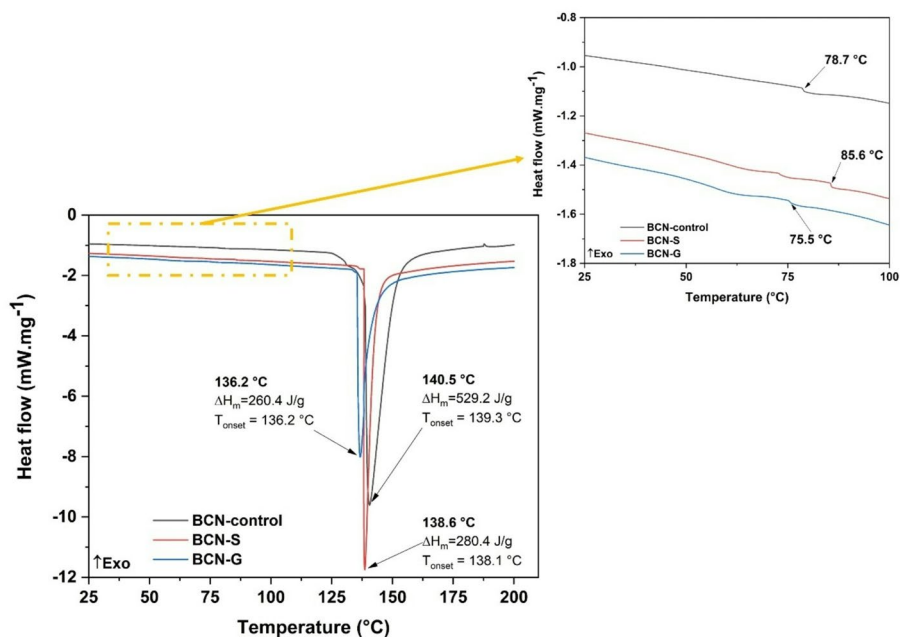


Fig. 6 DSC spectra and *inset* of BCN produced from different carbon sources: sucrose (traditional, BCN – control), soursop AIW (BCN-S), and genipap AIW (BCN-G)

ture medium components, or bioactive compounds that were not eliminated during the purification process [83]. These impurities can act as defects within the ordered crystalline domains, thereby influencing the overall thermal stability and reducing the energy required for melting. Such defects can indeed compromise the material's thermodynamic phase diagram [91]. Furthermore, the broader peaks observed in the DSC thermograms of BCN-control and BCN-S (when correlated with Table 5) could also reflect the higher concentration of these impurities or structural heterogeneities. Although crystalline cellulose usually exhibits higher thermal stability due to stronger intermolecular interactions and more ordered packing [50]. The thermal behavior observed in this stage cannot be attributed solely to crystallinity and may also be influenced by the presence of impurities and low-molecular-weight compounds derived from the substrates.

Analysis of the BCN-G on flexible film

The incorporation of composite agents, such as bacterial cellulose, into flexible films has been shown to enhance their performance [92]. The same applies to the development of new materials [17, 34, 35]. According to Wang et al. [92], bio-based nanomaterials such as bacterial cellulose have various applications in food packaging, including barrier and mechanical properties, as well as bioactive functions such as antioxidants and antimicrobials. In this context, the ability of bacterial cellulose nanofibers obtained from genipap waste (BCN-G) to improve optical barrier properties and water vapor permeability (WVP) was evaluated. BCN-G was selected for film preparation because it exhibited the most suitable combination of yield, crystallinity, and nanofiber morphology among the evaluated samples.

As shown in Fig. 7; Table 7, the films with BCN-G were evaluated for transparency. Their transmission and UV radiation shielding properties were also assessed (Table 8).

As shown in Table 7, the transparency and opacity values of the films demonstrate that interactions between cellulose composites and the polymer matrix influence the material's optical characteristics. This resulted in materials with a lower degree of transparency. However, light transmission levels were higher for BCN-15 than for the other treatments. This behavior may be associated with the formation of crystalline cellulose sites, as discussed earlier [93], see Table 5. Additionally, the type of culture medium and symbiotic culture used are intrinsic factors in the process that

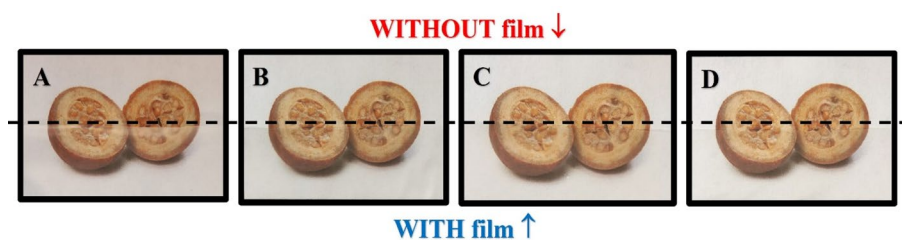


Fig. 7 Image showing the transparency of flexible starch films with different concentrations of BCN-G, where (A) starch film (control), (B) A-BCN-5, (C) A-BCN-10, (D) A-BCN-15

Table 7 Optical properties (transparency and opacity) of flexible starch films with different concentrations of BCN-G: 5% (BCN-5), 10% (BCN-10), and 15% (BCN-15)

Treatment	%T ₆₀₀	Transparency ¹ (%T/mm)	Abs ₅₀₀	Opacity ² (Abs.mm)
A-control	38.2	42.7 ± 0.1 ^a	0.432	0.016 ± 0.001 ^b
A-BCN-5	26.2	37.3 ± 2.0 ^c	0.595	0.023 ± 0.003 ^a
A-BCN-10	34.9	40.8 ± 0.1 ^{ab}	0.470	0.018 ± 0.001 ^b
A-BCN-15	41.1	39.7 ± 0.5 ^{bc}	0.400	0.016 ± 0.001 ^b

%T₆₀₀ is the percentage of transmittance at 600 nm; (²) Abs₅₀₀, absorbance at 500 nm. Means of the same column classified by different lowercase letters indicate that results differ significantly by the Tukey test at the 5% level ($n=3$)

Table 8 Light transmission (%) and UV protection (%) of flexible starch films (A-control) with different concentrations of BCN-G: 5% (BCN-5), 10% (BCN-10), and 15% (BCN-15), in different UV wavelength regions

Treatment	Transmittance (%)			UV-shielding (%)		
	UV-C*	UV-B**	UV-A***	UV-C	UV-B	UV-A
A-control	0.57 ± 0.03 ^b	0.70 ± 0.03 ^b	0.70 ± 0.01 ^a	99.43 ± 0.03 ^b	99.30 ± 0.03 ^b	99.30 ± 0.03 ^a
A-BCN-5	0.42 ± 0.07 ^a	0.51 ± 0.09 ^a	0.67 ± 0.20 ^a	99.58 ± 0.07 ^a	99.49 ± 0.09 ^a	99.33 ± 0.20 ^a
A-BCN-10	0.55 ± 0.02 ^b	0.66 ± 0.03 ^b	0.65 ± 0.03 ^a	99.45 ± 0.02 ^b	99.34 ± 0.03 ^b	99.35 ± 0.03 ^a
A-BCN-15	0.62 ± 0.02 ^b	0.75 ± 0.03 ^b	0.75 ± 0.03 ^a	99.38 ± 0.02 ^b	99.25 ± 0.03 ^b	99.25 ± 0.03 ^a

(*) UV-C region: 200–280 nm; (**) UV-B region: 280–315 nm; and (***) UV-A region: 315–400 nm. Means of the same column classified by different lowercase letters indicate that results differ significantly by the Tukey test at the 5% level ($n=3$)

can lead to changes in the composite's crystallographic properties [21, 85, 86], as illustrated in Fig. 4; Table 5. The transparency values of the films are less than 50%, which does not interfere with visibility of the internal content (Fig. 7). The low light transmission indicates an improvement in light barrier properties, such as protection against UV radiation (Table 8).

As shown in Table 8, all films exhibited excellent UV radiation barrier properties (>99% in tested regions). In the UV-A region, there was no significant difference between the treatments ($P>0.05$). However, in the UV-B and UV-C regions, the incorporation of BCN differed statistically ($P<0.05$) at 5% (v/v) only, improving the barrier properties in the more energetic regions. This behavior may be associated with inter- and intramolecular interactions in the composite-polymer matrix through hydrogen bonds, creating crystalline domains that obstruct light transmission [93, 94]. Our research successfully produced a flexible film that protects against more energetic UV regions (see Table 8), while maintaining the transparency of the material (see Fig. 7). Considering that the literature shows many organic UV radiation filters do not absorb more energetic radiation and are more specific to UV-A radiation [17, 94].

To preserve packaged foods more effectively, it is important to minimize the moisture content of the contents, as increased moisture can accelerate the growth of spoilage microorganisms and biochemical degradation reactions [95, 96]. Table 9 shows the water vapor barrier properties of flexible films.

The addition of BCN-G to the starch film polymer matrix is numerically lower for BCN-15, but not statistically significant ($P>0.05$) (see Table 9). However, the

Table 9 Thickness, water vapor permeability rate (WVPR), and water vapor permeability (WVP) of the flexible starch films with different concentrations of BCN-G: 5% (BCN-5), 10% (BCN-10), and 15% (BCN-15)

Treatment	Thickness (mm)	WVPR (g/h.m ²)	WVP (g.mm/kPa.h.m ²)
A-control	0.043 ± 0.000 ^b	80.13 ± 0.79 ^b	1.36 ± 0.02 ^a
A-BCN-5	0.045 ± 0.001 ^b	79.07 ± 1.65 ^b	1.40 ± 0.10 ^a
A-BCN-10	0.056 ± 0.006 ^a	69.91 ± 0.71 ^a	1.49 ± 0.16 ^a
A-BCN-15	0.041 ± 0.001 ^b	77.24 ± 2.84 ^b	1.24 ± 0.08 ^a

Means of the same column classified by different lowercase letters indicate that results differ significantly by the Tukey test at the 5% level ($n=5$)

film containing 10% (v/v) BCN-G differed in thickness and WVTR compared to the others ($P<0.05$). This variation may be due to the cellulosic material being dispersed unevenly in the polymer matrix during homogenization, or due to unevenness in the drying surface [97, 98]. Betlej et al. [97] showed in their studies with native and modified bacterial cellulose that variations in the WVP of films may be related to the distribution in the material, the density and the spatial structure of the BC material.

In terms of permeability, all treatments had a higher average than the control except for the 15% (v/v) BCN-G treatment. It is believed that the greater incorporation of the highly crystalline composite (Table 5) created obstacles or zones where water molecules could not diffuse [99]. Literature shows that polymer–BCN interactions and/or inter- and intra-chain interactions through hydrogen bonds in the crystalline regions of BCN can reduce water vapor diffusivity through micropores in the material by forming a compact network of nanofibers [100–102]. In their studies with bacterial cellulose/poly(vinyl alcohol), Li et al. [99] observed that incorporating crystalline compounds or inducing crystallization reduces WVP. The authors attribute this to a reduction in amorphous spaces and the formation of ‘obstacles’ due to the presence of the crystalline fraction, which restricts molecular mobility.

Conclusions

The use of agro-industrial waste (AIW) as alternative fermentable carbon sources, particularly from Amazonian tropical fruits like soursop (*Annona muricata* L.) and genipap (*Genipa americana* L.), has demonstrated its feasibility, sustainability, and efficacy in producing bacterial cellulose nanofibers (BCN). Integrating X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analyses was crucial for a deeper understanding of how the carbon source influences the material’s physico-chemical properties. Notably, the cellulose produced from genipap waste (BCN-G) stood out, exhibiting a crystallinity index (CrI) of 75.2% and a crystallite size (D_{av}) of 163.1 nm. These structural characteristics are highly relevant to the packaging sector, as they confer improved material properties, including mechanical strength, gas barrier capabilities, optical clarity, water retention capacity, and biocompatibility, particularly with the incorporation of bioactive compounds. Although the control BCN exhibited a higher fusion enthalpy ($\Delta H_m = 529.2$ J/g), indicating greater stability in its crystalline domains, this study reinforces the potential of AIW. The incorporation

of BCN-G significantly improved the functional performance of starch-based films, particularly by reducing light transmission, enhancing UV-shielding, and decreasing water vapor permeability, which are critical attributes for active and sustainable food packaging materials. These improvements are crucial for the packaging sector, as the material can be used to extend the shelf life of food products. The BCN, especially derived from genipap residue, represents a promising, versatile, and biodegradable material capable of replacing conventional polymers, thereby contributing significantly to the sustainability of the food industry and reducing environmental impact.

This work validates the strategy of utilizing renewable sources, aligning with the principles of the circular economy and Sustainable Development Goal (SDG) 12: Responsible Consumption and Production. Beyond confirming the viability of AIW for BCN production, this research lays the groundwork for potential industrial-scale application. Future advances should encompass optimizing BCN synthesis and functionalization through the isolation and next-generation sequencing (NGS) of microbial communities and conducting more comprehensive analyses such as Nuclear Magnetic Resonance (NMR) and Atomic Force Microscopy (AFM). In addition, further studies should evaluate the mechanical and antimicrobial properties of the developed films, which were beyond the scope of the present work but are essential for advancing their application in food packaging systems.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict interest No conflict of interest exists in the submission of the manuscript.

Ethical approval Not applicable.

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